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Research Article

Morphological and molecular characterization of *Halamphora lukasiewiczii* sp. nov. (Bacillariophyceae, Amphipleuraceae), a new extremophilic diatom from petroleum seeps in the Polish Carpathians

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Abstract

Crude oil is toxic in high concentrations. However, several microalgae are able to proliferate in these extreme environments. In this study, we describe diatoms in natural oil seeps in the Polish Carpathians. Only two species were found: *Halamphora lukasiewiczii* **sp. nov.** and *Navicula salinarum*. Cultures were established and characterized using morphological, and molecular data based on the *rbc*L gene with secondary support from SSU rRNA sequences. *Halamphora lukasiewiczii* **sp. nov.** was examined using light, fluorescence, and scanning electron microscopy. This new species is closely related to *Halamphora aponina*, *H. angustiformis*, *H. borealis*, *H. kenderoviana*, *H. witkowskii*, and *H. margalefii* var. *lacustris*. It is distinguished by small, dorsiventral valves (8.0–22.5 µm long, 3.0–5.0 µm wide) with exceptionally dense ventral striation (approximately 55 by 10 µm), the highest among related taxa. The H-shaped chloroplast is located ventrally, with lobes extending below the valves and forming a characteristic U- to V-shaped outline in apical view. In molecular phylogeny, *Halamphora lukasiewiczii* **sp. nov.** is closely related to *H. margalefii*, differing by 13 base pairs for a length of 1288 bp in the *rbc*L gene sequence of *H. lukasiewiczii* SZCZ MR2353. The DNA sequences of the inland Polish strain of *Navicula salinarum* showed low genetic divergence from the available sequences from Chinese coastal waters. The adaptations that allow *Halamphora lukasiewiczii* **sp. nov.** and *Navicula salinarum* to proliferate in this extreme environment require further investigation.

Key words: Benthic diatom, extreme environments, hydrocarbon seep, morphology, *Navicula salinarum*, phylogeny, *rbc*L, SSU rRNA

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Introduction

Diatoms occupy a variety of environments on Earth where they can find water, light, and nutrients, especially silica for their frustules. These environments include almost all aquatic and wet terrestrial habitats like open oceans and marine coasts, lakes, rivers, swamps and moors, but also ice surfaces, hot springs, soils, tree trunks, or any wet surfaces, natural or synthetic, including plants and animals. Some habitats are inhospitable to the majority of organisms, and these are referred to as extreme environments. In those near to the extreme ranges of environmental variables only highly adapted forms of life can exist. An extreme environment refers to one where physicochemical parameters are outside the range of tolerance for most organisms. This may include very high or very low temperatures, pH values, salinity, dryness, high concentration of heavy metals, very high or low levels of radiation, and, to a certain extent, anaerobic environments (Seckbach et al. 2013). The microorganisms occurring in these environments are defined as extremophiles.

Natural oil seeps are one example of an extreme environment. Also referred to as petroleum or hydrocarbon seeps, these are naturally occurring places on land where oil or other forms of petroleum escape from deep reservoirs. When oil seeps to the surface, it undergoes various stages of decomposition including vaporization of the light-end hydrocarbons, microbial degradation, polymerization, and oxidation. This decomposition tends to thicken the migrating oil, and liquid oil is gradually converted to asphalt and asphaltite (Khilyuk et al. 2000). Fresh crude oil is toxic to microalgae at high concentrations, but studies of natural hydrocarbon seeps occurring on sea floors show that they can have either inhibiting or stimulating effects at low concentrations. Temperature and nutrient profiles above seep sites suggest that nutrient-rich water upwells from the depths that may facilitate microalgae growth (D'souza et al. 2016).

There is little data about pro- and eukaryotic algae, including diatoms, that are able to survive directly in terrestrial petroleum seeps (Baker et al. 2022). Most data about microorganisms associated with these habitats refer to sulfate-reducing bacteria isolated from oil reservoir samples, mostly offshore or marine (e.g., Leu et al. 1999; Kaster et al. 2009; Korenblum et al. 2012).

In this paper we aim to shed light on this interesting, unique habitat, with the description of a diatom species new to science: *Halamphora lukasiewiczii* sp. nov. We identified this diatom in two separate natural oil seeps located in southeastern Poland. This paper presents morphological and molecular data based on monoclonal cultures. The new species is compared to published sources in terms of morphology and molecular phylogeny (*rbc*L and SSU rRNA), and its similarities to known species are discussed.

Materials and methods

Abbreviations

bp, base pairs; **DAPI**, 4',6-diamidino-2-phenylindole; **DMSO**, dimethyl sulfoxide; **LM**, light microscopy; **ML**, maximum likelihood; **PBS**, phosphate-buffered saline; **PCR**, polymerase chain reaction; ***rbc*L**, ribulose-1,5-bisphosphate carboxylase large subunit; **SEM**, scanning electron microscopy; **SSU rRNA**, small subunit ribosomal RNA.

Study area and sampling

The study area was located in southeast Poland (Fig. 1). Water and diatom samples at site 1 and 2 were collected in August and October 2024. The distance between the two sites is about 20 km. Water conductivity, pH, and temperature were measured in the field with a WTW ProfiLine pH/Cond 3320 multiparameter meter (Xylem Analytics, Weilheim, Germany).

Site number 1 is located in the village of Uherce Mineralne, on a meadow grazed by horses (49°27'10.2"N, 22°24'58.9"E). Oil outflow was also accompanied by the natural leakage of mineral water and gas. The area of pond created by natural petroleum outflow was about 7 m^2. The pond was filled with water covered by a thin layer (1–3 mm) of petroleum (Fig. 1C). The pond bottom substrate was composed of sand sediments (meadow soil). During strong winds, a petroleum layer partially recedes, exposing the water surface. Water samples for

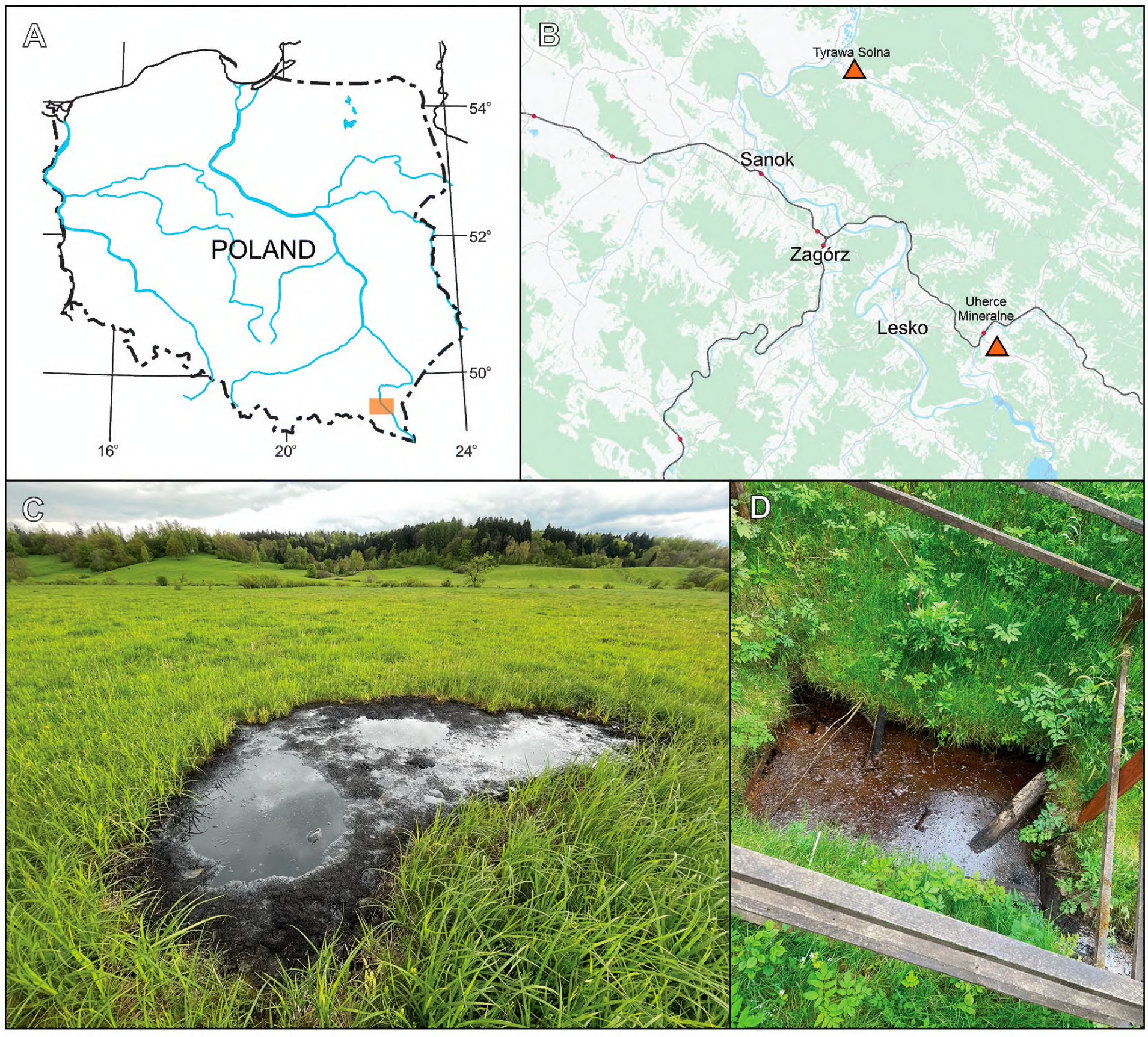

Figure 1. Sampling sites. **A.** Location of study area (marked by orange rectangle); **B.** Location of sampling sites in Uherce Mineralne (site 1) and Tyrawa Solna (site 2) marked by orange triangles; **C.** Site 1 – natural petroleum seep; **D.** Site 2 – brine and petroleum spring in an abandoned well.

physicochemical measurements were collected from the water column, whereas diatom samples were collected from sediments. Water and diatoms were collected using single-use plastic pipettes. The depth of pond at the sampling site was approximately 5–10 cm. Measurements were taken of electric conductivity (467–518 µS × cm^{-1}), pH (7.0–7.1), and temperature (20.3–26.9 °C).

Site number 2 is a brine spring located in an abandoned well (49°36'14.1"N, 22°16'48.5"E) within the village of Tyrawa Solna, on the border of a forest and cultivated fields. In addition to highly saline water, fresh crude oil was also seeping from the spring. Until the beginning of the twentieth century, the spring had been used by local inhabitants for salt production. Currently, the spring is abandoned and secured with beams (Fig. 1D). The surface area is about 1 m^2. Diatom samples were collected by scratching using plastic pipettes from wooden beams supporting the sides of the well. Water samples for physicochemical measurements were taken from petroleum-free areas and below the petroleum surface using single-use plastic pipette. Measurements were taken of electric conductivity (75400–81000 µS × cm^{-1}), pH (6.6–6.7), and temperature (13.3–16.8 °C).

Culturing methods

Cell cultures were obtained by pre-incubating portions of the material collected (2 mL) in f/2 culture medium (Guillard 1975). Next, monoclonal cultures were established by isolating single diatom cells using single-use plastic pipette under an inverted light microscope Zeiss ID-03 (Carl Zeiss, Jena, Germany) at 20× and 40× objectives. The cells were transferred into plastic Petri dishes with f/2 culture medium. Two types of medium; freshwater and marine (with a salinity of 35 psu) were used to check the ability of diatoms to grow in each salinity. Diatoms were cultivated at 20 °C under a 12:12 photoperiod. Three strains, cultured on marine medium, were selected for further analyses:

- RZ06 – obtained from the natural petroleum seep at site 1;
- RZ037– obtained from the abandoned petroleum-rich well at site 2;
- RZ81 – obtained from the abandoned petroleum-rich well at site 2.

Microscopy analysis

Diatom slides were prepared by boiling samples in 30% hydrogen peroxide (H_2O_2) for about 3 hours. Cleaned diatom material was pipetted onto coverslips and dried, then mounted on glass slides with Naphrax mounting medium (Brunel Microscopes Ltd, Wiltshire, UK). Light microscope (LM) observations of living cells and cleaned frustules were made with a Nikon Eclipse 80i (Nikon Corporation, Tokyo, Japan) microscope equipped with Differential Interference Contrast (DIC) and a 100× Plan Apochromatic oil immersion objective. For SEM (Scanning Electron Microscopy), a few drops of material were placed on 5 µm pore size Whatman Nuclepore polycarbonate membrane filters (Fisher Scientific, Schwerte, Germany). Once air-dried in room temperature, the membranes were mounted on aluminum stubs and coated with 20 nm of gold using a turbo-pumped Quorum Q 150 T ES coater (Judges Scientific plc, London, UK). SEM observations were performed with a Hitachi microscope SU8010 (Hitachi Ltd, Tokyo, Japan). Diatom terminology followed Round et al. (1990) and Levkov (2009).

Fluorescence microscopy analysis

Diatom cell nuclei were stained with 4',6-diamidino-2- phenylindole (DAPI). Cells were gently centrifuged (1500 rpm; 2 minutes), washed twice with PBS buffer, and resuspended in PBS buffer. DAPI was added to a final concentration of 2 µg/mL (100 µg/mL stock solution in Milli Q water) and incubated for 10 minutes. Fluorescence imaging was done with either a 40× (UPlanFL; numerical aperture (NA) 0.75; Olympus) or a 100× (UPlanFL; NA 1.30; Olympus) objective lens coupled with a band pass (BP) 360/370 nm excitation filter.

Cell vacuole membranes were stained with MDY-64 (Life Technologies, Eugene, OR, USA). The cells were gently centrifuged (1500 rpm; 2 minutes), washed twice with PBS buffer, and resuspended in 20 mM HEPES buffer pH 7.4. MDY-64 was added to a final concentration of 10 µM (10 µM stock solution in DMSO) and incubated for 15 minutes. Fluorescence imaging was done with a 40× (UPlanFL; NA 0.75; Olympus) objective lens with a BP 470/490 nm excitation filter.

The autofluorescence emitted by chlorophyll molecules was observed with a 40× (UPlanFL; NA 0.75; Olympus) objective lens with a BP 470/490 nm excitation filter. Microscopic images were taken with an Olympus BX-51 microscope equipped with a DP-72 digital camera and cellSens Dimension v1.0 software.

DNA isolation, amplification, and sequencing

Genomic DNA was extracted after centrifugation of the strains RZ06, RZ037, and RZ81 using a 10% working solution of Chelex resin (canister #1421253, Bio-Rad, Hercules, CA, USA). Chelex working solution (200 µL) was added to each pellet in 1.5 µL Eppendorf tubes, vortexed, heated for 20 min at 95 °C, and centrifuged for 5 min at 10000 rpm (modified from Dąbek et al. 2017). Isolated DNA was transferred to Eppendorf tubes and stored frozen at -21 °C.

The nuclear SSU rRNA and chloroplast *rbc*L genes were amplified following the protocol from Ashworth et al. (2013) using a S1000TM Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR products were sequenced in both directions with the Sanger method at SEQme (SEQme s.r.o., Dobříš, Czech Republic). The obtained sequences of SSU rRNA from RZ81 (*Navicula salinarum* Grunow) and *rbc*L from RZ06, RZ37, and RZ81 (both *Halamphora lukasiewiczii* sp. nov. and *N. salinarum*) were assembled with BioEdit ver. 7.2.5 (Hall 1999). In the case of SSU rRNA sequences of RZ06 and RZ37 the forward and reverse reads could not be aligned and only one fragment of each strain was used for the phylogeny. The sequences used in phylogenetic analyses were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with following accession numbers (SSU rRNA and *rbc*L, respectively) MR2353 (=RZ06): PX576035, PV696445; MR2632 (=RZ037): PX576036, PV696446; RZ81: PX576037, PX521590.

In order to calculate a maximum likelihood (ML) phylogeny, independent phylogenetic trees were built based on SSU rRNA and *rbc*L genes for species ascribed in GenBank as belonging to the genus *Halamphora*, and to the genus *Navicula*. In the case of *Halamphora*, the tree based on concatenated SSU rRNA and *rbc*L datasets were built. Prior to computing ML analysis, each dataset was aligned independently using MAFFT 7 (Katoh and Standley 2013) and trimmed automatically with trimAl (Capella-Gutiérrez et al. 2009). The best evolutionary model was chosen using ModelTest-NG (Darriba et al. 2020) for each dataset independently.

For both SSU rRNA and *rbc*L of *Halamphora* the model was TIM3+I+G4, for SSU rRNA of *Navicula* TIM3+I+G4 and for *rbc*L of *Navicula* TrN+I+G4. Alignments were then concatenated with Phyutility (Smith and Dunn 2008). The resulting fasta files were used for calculating ML phylogenetic trees with 1000 ultrafast bootstrap replicates using IQ-TREE 2.2.0 (Minh et al. 2020). The phylogenetic trees were created with iTOL online software v7.4.2 (Letunic and Bork 2024).

Results

Molecular phylogeny

The tree topology inferred from the dataset of *rbc*L (Fig. 2) recovered both *H. lukasiewiczii* sp. nov. strains (red frame) in a monophyletic clade with bootstrap probability of 99%. Both sequences are identical for a length of

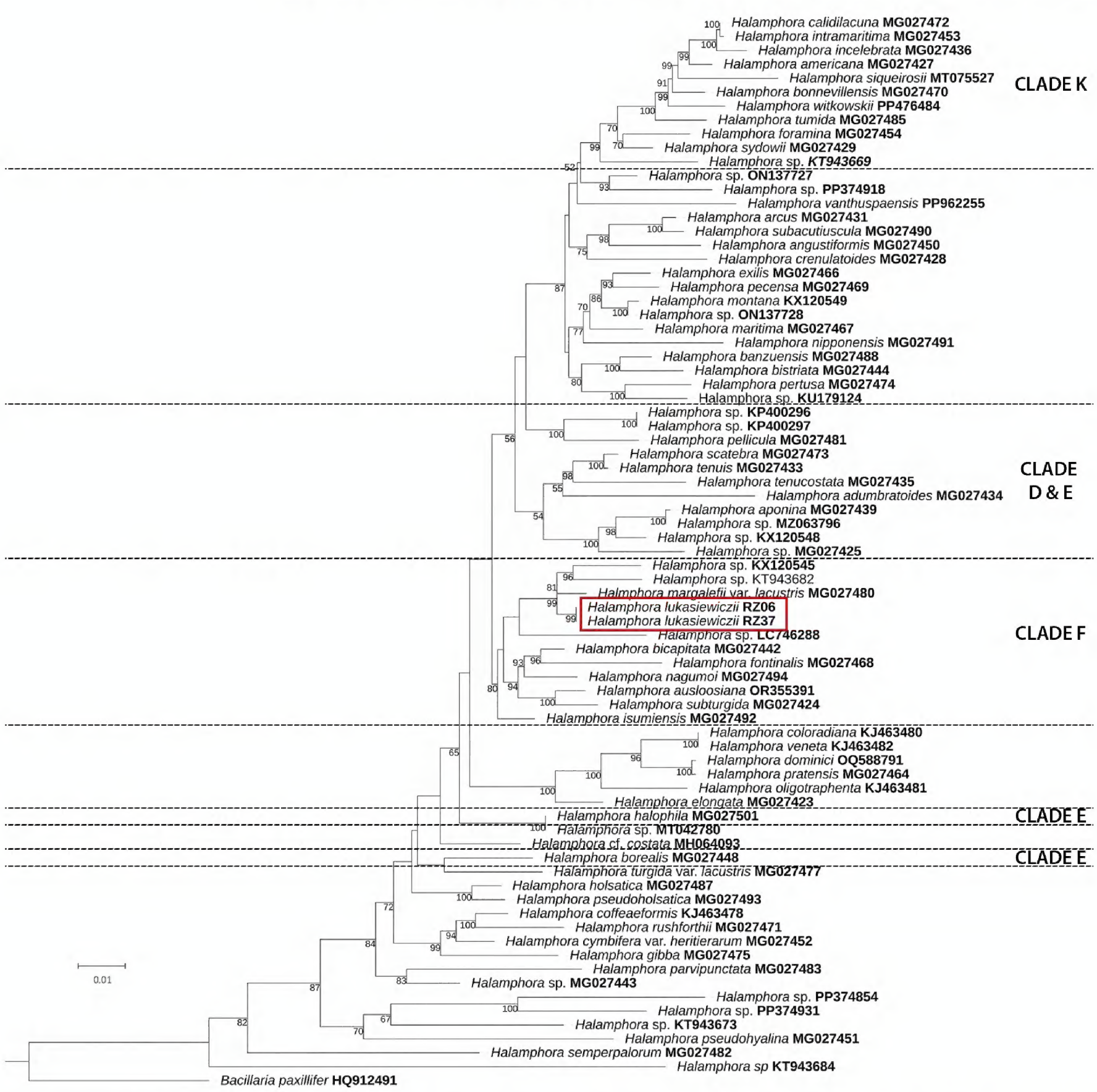

Figure 2. Maximum likelihood phylogenetic tree inferred from *rbc*L gene sequences. Sequences belonging to *Halamphora lukasiewiczii* sp. nov. are indicated by box. ML bootstrap support (BP) below 50 are omitted. The scale bar represents 0.01 substitutions per site.

868 bp, corresponding to the length of the shorter sequence (SZCZ MR2632). *Halamphora lukasiewiczii* sp. nov. is sister to a clade that contains *H. margalefii* var. *lacustris* (P.Sánchez) J.G.Štěpánek & Kociolek and unidentified species of *Halamphora* KX120545 and KT943682 with BP 99%. In terms of sequence divergence, *H. lukasiewiczii* sp. nov. differs from *H. margalefii* var. *lacustris* by 13 base pairs over a length of 1288 bp in the *rbc*L gene sequence of strain SZCZ MR2353, whereas the *Halamphora* sequences KX120545 and KT943682 differ by 26 bp over 1324 bp and by 21 bp over 1152 bp, respectively. This clade appears in a larger cluster composed of 7 more *Halamphora* species: *H. bicapitata* (M.H.Hohn & J.Hellerman) J.G.Štěpánek & Kociolek, *H. fontinalis* (Hustedt) Levkov, *H. nagumoi* J.G.Štěpánek, Mayama & Kociolek, *H. ausloosiana* Van de Vijver & Kopalová, *H. subturgida* (Hustedt) Levkov, *H. isumiensis* J.G.Štěpánek, Mayama & Kociolek, and one unidentified species of *Halamphora* (LC746288). The tree based on SSU rRNA is presented in Suppl. material 1: fig S1 and the tree based on concatenated dataset of SSU rRNA and *rbc*L is presented in Suppl. material 1: fig S2.

The *rbc*L gene sequence of *Navicula salinarum* RZ81 (Fig. 3) formed a sister group with sequences of *Navicula* sp. (MT432483, BP 84%), and *N. salinarum* (KY320303), *N. salinarum* f. *minima* (KY320304), *Navicula* cf. *salinarum* (KY320293), and *N. trivialis* Lange-Bertalot (KY320311) with high bootstrap probability (98%). The tree based on SSU rRNA is presented in Suppl. material 1: fig S3. The list of sequences used can be found in Suppl. material 2.

Taxonomy

Phylum: Heterokontophyta Moestrup, R.A.Anderson & Guiry, 2023
Class: Bacillariophyceae Haeckel, 1878
Order: Naviculales Bessey, 1907
Family: Amphipleuraceae Grunow, 1862
Genus: *Halamphora* (Cleve) Levkov, 2009

***Halamphora lukasiewiczii* M.Rybak, Peszek, Górecka, Zadrąg-Tęcza, Levkov, sp. nov.**
Figs 4–8

Description. Valve length 8.0–22.5 µm, valve width 3.0–5.0 µm (n = 100) (Fig. 4). Valves narrow, semi-lanceolate, dorsiventral with arched dorsal margin and straight to weakly tumid ventral margin (Fig. 4). Valve ends protracted, subcapitate to capitate (Figs 4, 5A, B). Axial area narrow (Figs 5A–E, 6A–D). Central area absent on dorsal side, ventral side small, bordered by 4 to 6 shortened striae (Figs 5A–E, 6A–D). Raphe filiform and distally curved toward dorsal side (Figs 5A–D, 6A–C). Proximal raphe ends slightly expanded within larger surface depression (Figs 5A–E, 6A–D). Internally, distal raphe ends slightly deflected ventrally and terminated with helictoglossae (Figs 5H (arrow), 6H). Internally, proximal raphe ends terminated with fused central helictoglossae (Figs 5G, 6E–G). Dorsal striae fine (24–30 in 10 µm), almost parallel in middle, becoming slightly radiate towards valve apices (Fig. 4). Dorsal striae biseriate in most of cells, or occasionally partly uniseriate (Figs 5A–D, 6B (arrows), D). Areolae small, irregularly shaped to round (Figs 5A, B, D, 6D). On ventral side

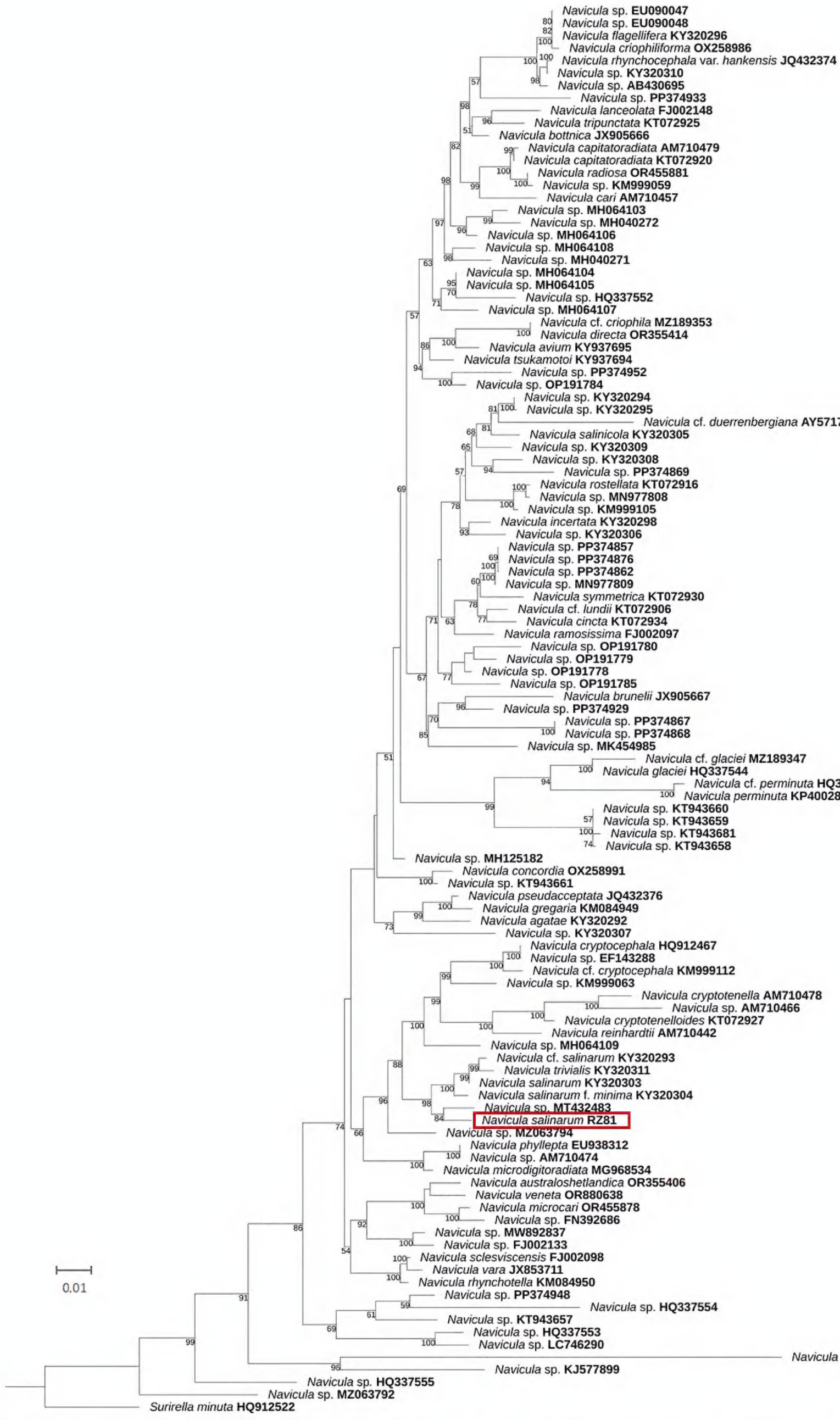

Figure 3. Maximum likelihood phylogenetic tree inferred from *rbc*L gene sequences. Sequence belonging to *Navicula salinarum* is indicated by box. ML bootstrap support (BP) below 50 are omitted. The scale bar represents 0.01 substitutions per site.

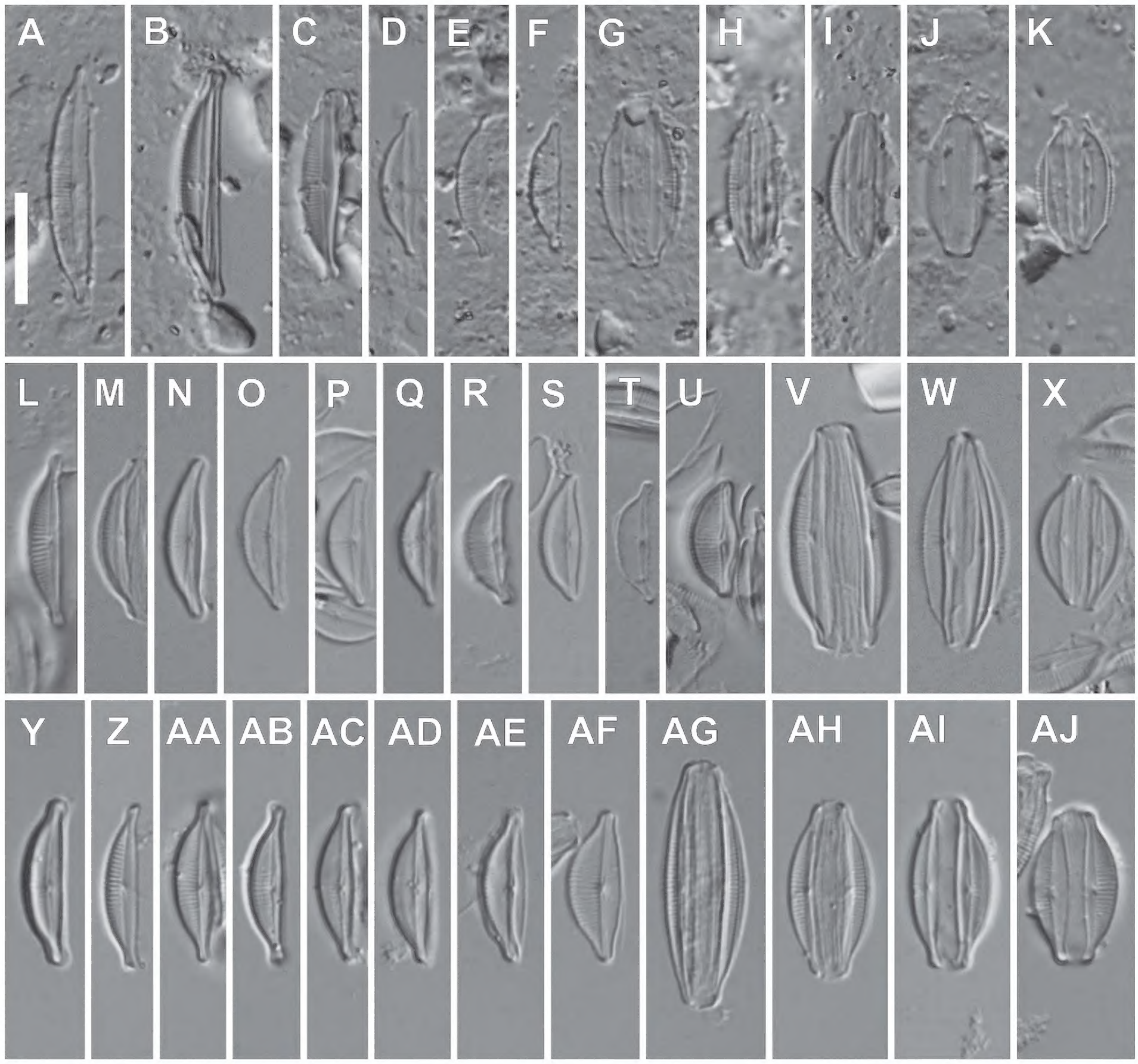

Figure 4. Light micrographs of *Halamphora lukasiewiczii* sp. nov. **A–K.** Valves from a wild sample collected at site 2 representing holotype population; **L–X.** Valves from culture sample, from site 1; **Y–AJ.** Valves from culture sample from site 2. Scale bar: 10 µm.

striae are uniseriate to biseriate, also with single row of longitudinal areolae, ca. 55 in 10 µm (Figs 5A, B (arrows), D (arrows), 6B). Internally, areolae on both sides occluded by hymene and separated by slightly thickened virgae (Fig. 6E–H). Raphe ledge (partial conopeum) well developed (sometimes reduced (Fig. 6A)), almost linear and weakly expanded in center and near valve ends; slightly elevated from valve surface on dorsal side (Figs 5A, C–E, 6A–D). Girdle bands numerous, each bearing two rows of large pores (Fig. 5F–H). Chloroplast single and H-shaped (Figs 7, 8A, B). Chloroplast isthmus located on ventral side, chloroplast lobes spread out directly under valves, giving a U- to V-shaped appearance in apical view (Fig. 8B (arrow)). Nucleus located slightly outside center of cell (Fig. 8D). Interior of cell mostly filled with large vacuoles (2–4), additional smaller vacuoles may be present at cell apices (Fig. 8C). No other organelles observed.

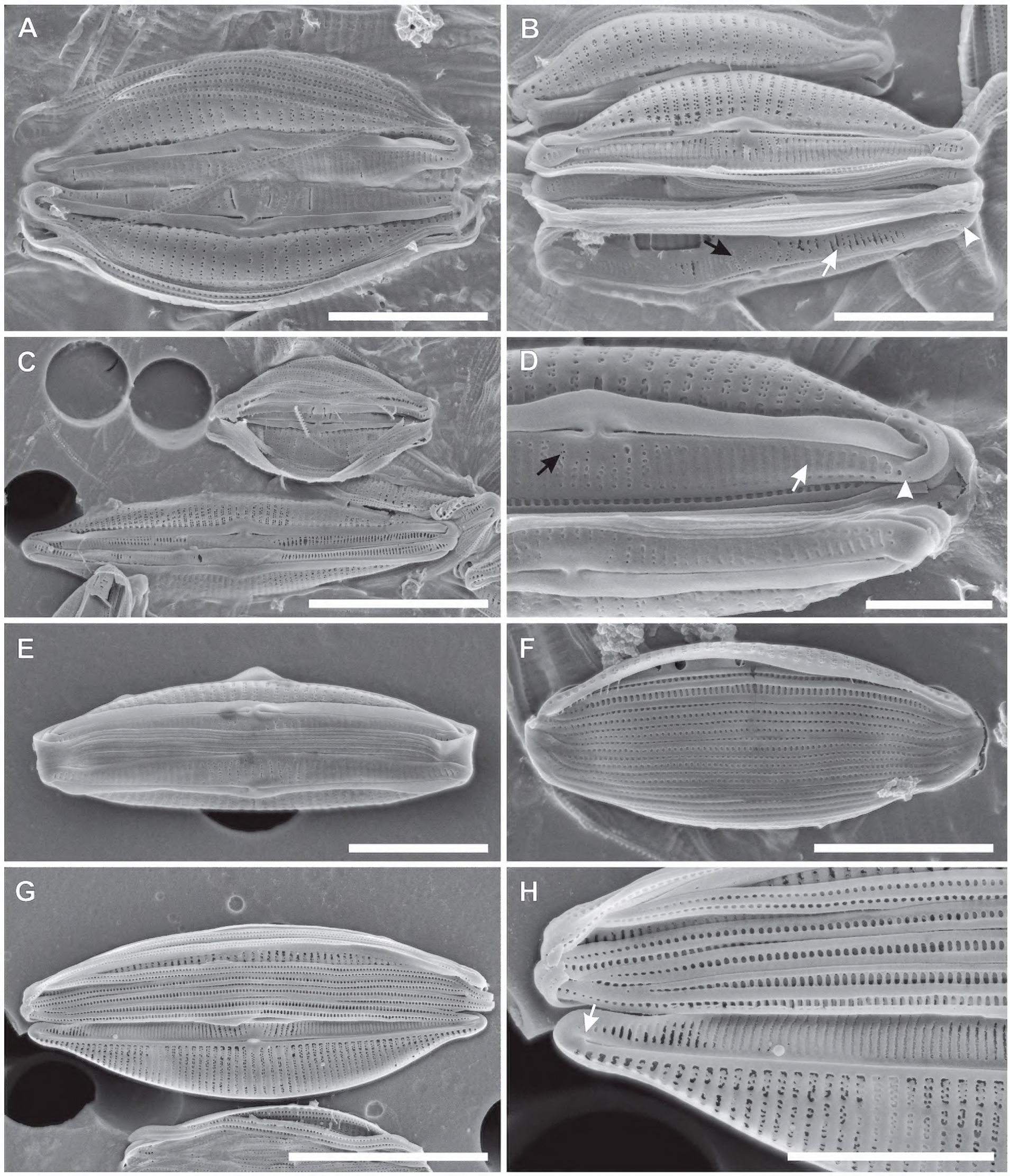

Figure 5. SEM images of *Halamphora lukasiewiczii* sp. nov. from a wild sample. **A–F.** External view of entire frustule; **B.** Valve with morphological variability of ventral striae. From single areola (arrowhead) at the valve apex to biseriate (black arrow), also with composed of single row of longitudinal areolae (white arrow); **C.** Two frustules of different sizes are visible in field of view; **D.** Enlargements of center and apex of valve, with variability of ventral striae – from single areolae (arrowhead) to single row of longitudinal areolae (white arrow) to rarely biseriate striae (black arrow); **F.** Frustule in dorsal view with numerous girdle bands; **G.** Internal view of valve; **H.** Internal view of valve apex with helictoglossae (white arrow) and numerous girdle bands. Scale bars: 5 µm (**A, B, E, F**); 2 µm (**D**); 10 µm (**C, G**); 4 µm (**H**).

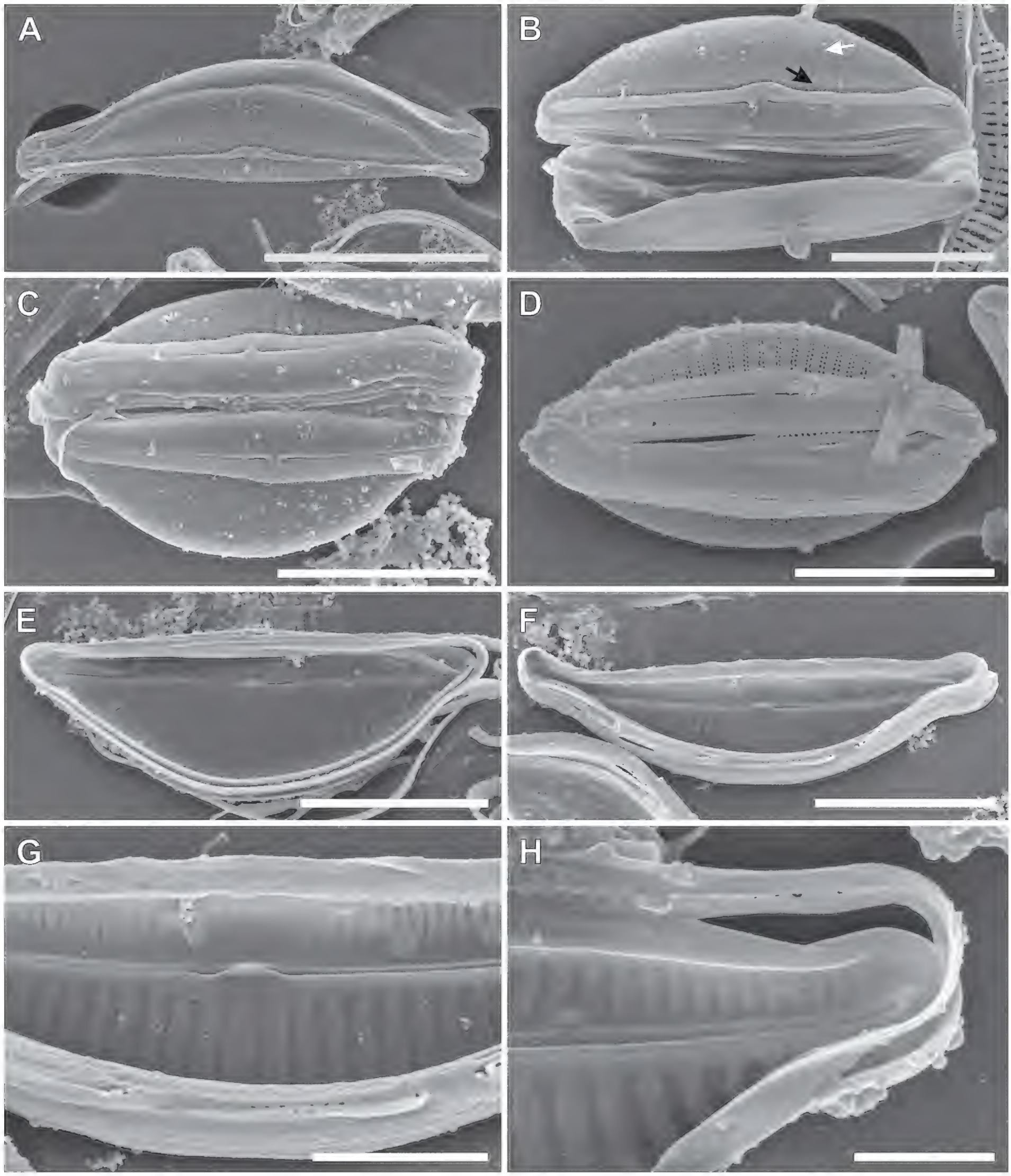

Figure 6. SEM images of *Halamphora lukasiewiczii* sp. nov. from culture. **A–D.** External view of the entire frustule; **B.** Valve with variable structure of striae. Form biseriate (black arrow) to uniseriate (white arrow); **E, F.** Internal view of entire valve; **G.** Internal view of central area and raphe endings; **H.** Internal view of valve apex and with helictoglossae. Scale bars: 10 µm (**A**); 5 µm (**B–F**); 2 µm (**G**); 1 µm (**H**).

Similar taxa. *Halamphora lukasiewiczii* sp. nov. is closely related to *H. angustiformis* J.G.Štěpánek & Kociolek, *H. aponina* (Kützing) Levkov, *H. borealis* (Kützing) Levkov, *H. kenderoviana* Zidarova, P.Ivanov, Dzhembekova,

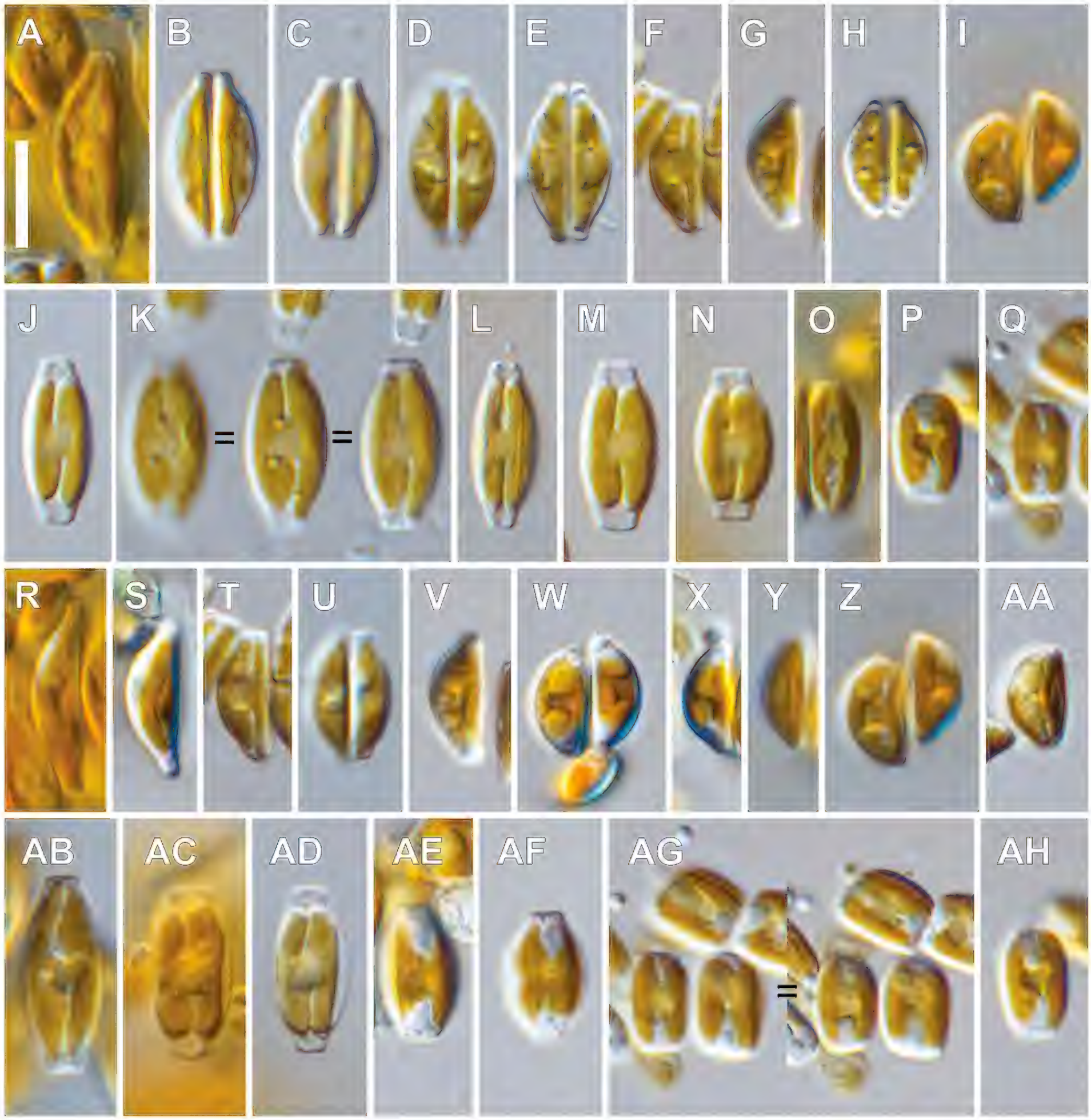

Figure 7. Light micrographs of living cultured *Halamphora lukasiewiczii* sp. nov. **A–Q.** culture from site 1; **R–AH.** Culture from site 2; **A–I, R–AA.** Size diminution series of valve views. **J–Q, AB–AH.** Frustules in ventral and dorsal views. Scale bar: 10 µm.

M. de Haan & Van de Vijver, *H. margalefii* var. *lacustris* (P.Sánchez) J.G.Štěpánek & Kociolek, and *H. witkowskii* Yilmaz, Solak & Gastineau. *Halamphora lukasiewiczii* sp. nov. is distinguished by small, dorsiventral valves (8.5–22.5 µm length, 3.0–5.0 µm width) with exceptionally dense ventral striation (ca. 55 in 10 µm), the highest among related taxa.

Type locality. Poland • Subcarpathian region, periphyton from wooden beams of abandoned well with brine and natural petroleum seep in Tyrawa Solna village, 49°36'14.1"N, 22°16'48.5"E, collected on 17 August 2024 by Mateusz Rybak and Łukasz Peszek.

Holotype. SZCZ MR2632 (permanent slide) with holotype population (Fig. 4A–K), deposited in Szczecin Diatom Collection of the University of Szczecin, Poland.

Isotype. Isotype 1 –slide under access number 2024/32 in the Diatom Strain collection in Herbarium of University of Rzeszów, Isotype 2 – permanent slide under accession number MKNDC 15068 in the Macedonian National Diatom Collection.

Etymology. The species epithet honor to Ignacy Łukasiewicz (1822–1882), an outstanding Polish pharmacist, chemist, pioneer of the oil industry, and social activist. Ignacy Łukasiewicz was the inventor of the kerosene lamp and one of the first to use oil distillation on an industrial scale.

Ecology and distribution. Currently, the species is known only from two locations in the Subcarpathian region in Poland, where it occurs in natural oil seeps. At site 1, the species was observed only after the material collected was incubated in f/2 medium. The species was not observed in the wild sample, which may have been due to its very rare occurrence at the site studied, the high concentration of petroleum, or the lack of sunlight blocked by the petroleum layer on the water surface (see Fig. 1C). In case of the outflow at site 2, the species was recorded in the natural sample and reaches around 70% share in assemblage. At this site, together with the species described, only one other diatom species, *Navicula salinarum* Grunow, co-occurred (Fig. 9). Since it occurred in an unusual habitat, proper species

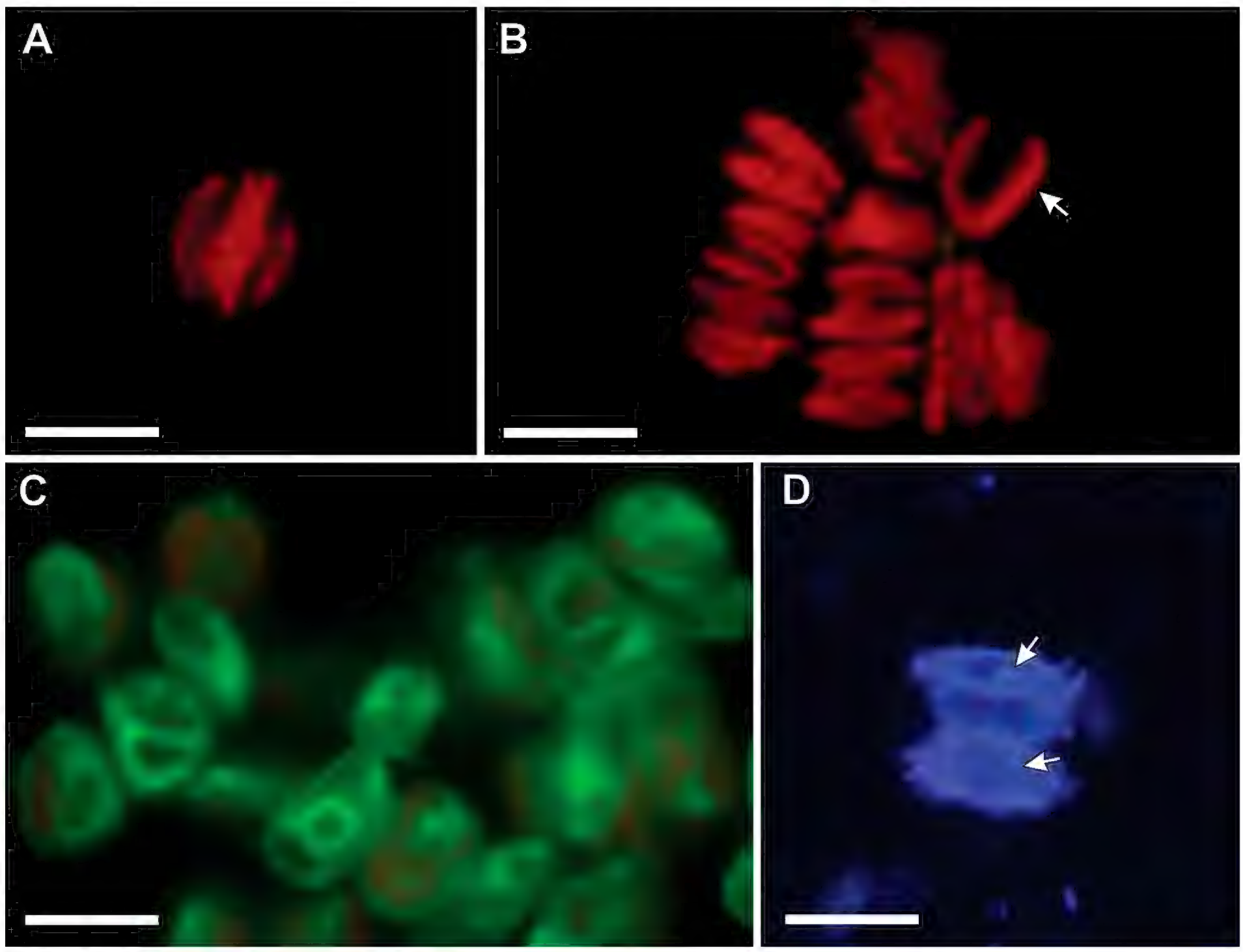

Figure 8. *Halamphora lukasiewiczii* sp. nov. Cells from culture sample, from site 1 observed under fluorescence microscopy; **A, B.** Cells with visible chlorophyll autofluorescence; **A.** Pair of daughter cells displaying visible H-shaped chloroplasts; **B.** Group of cells, arrows indicate cell in apical view and U-shaped chloroplasts; **C.** Group of cells with vacuole membranes stained using MDY-64 dye; **D.** Cells stained with DAPI dye, arrows indicates nucleus position. Scale bars: 10 µm.

identification of *N. salinarum* was confirmed by phylogenetic analysis (see Molecular Phylogeny section). Observed valve length was: 22.0–44.0 µm, valve width 8.0–12.0 µm, with 15–16 striae in 10 µm.

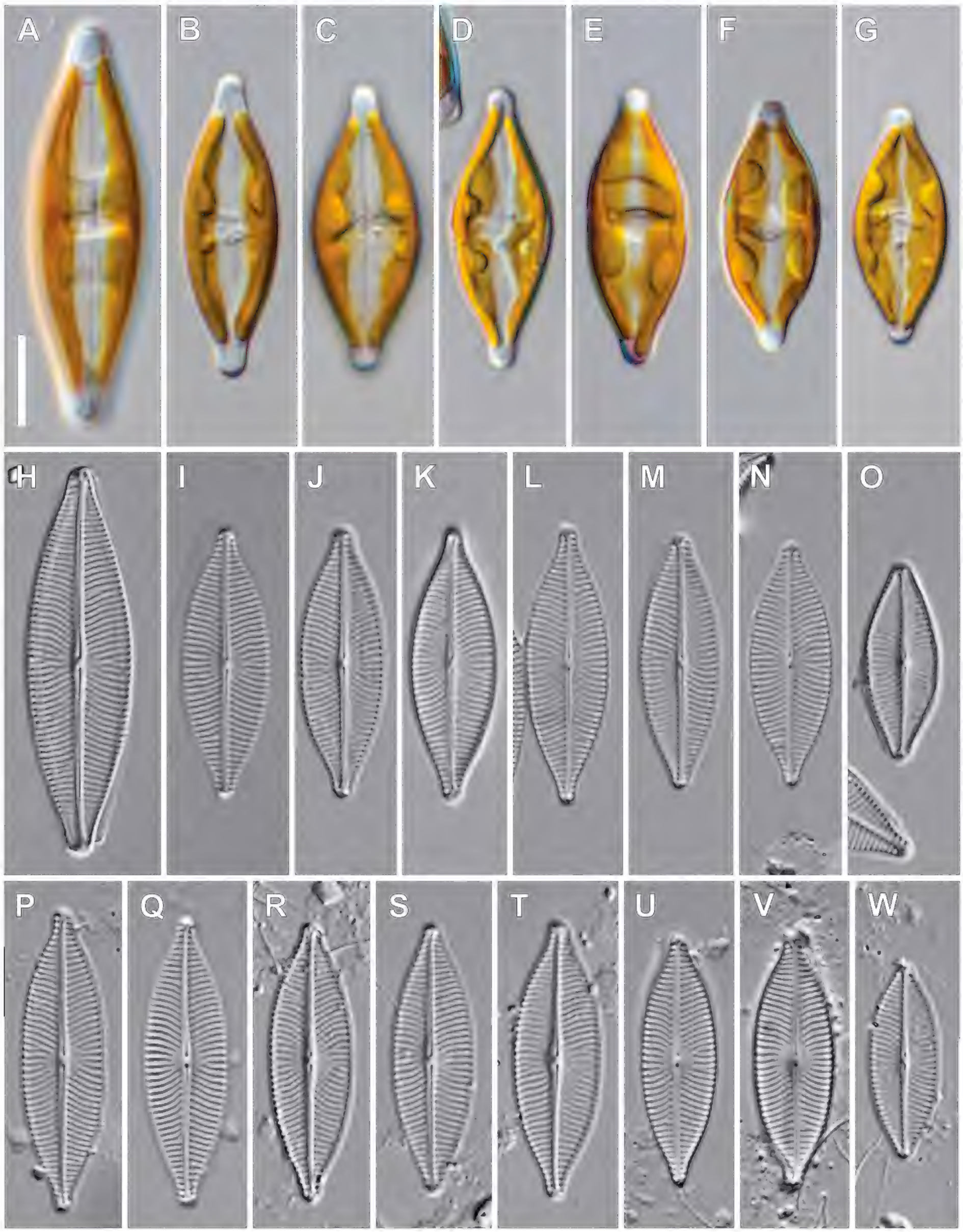

Figure 9. Light micrographs of *Navicula salinarum* from site number 2. **A–G.** Living cells from culture; **H–O.** Cleaned cells of *N. salinarum* from culture; **P–W.** Cleaned cells from wild material. Scale bar: 10 µm.

Discussion

The genus *Halamphora* (Cleve) Levkov for many years was combined with the genus *Amphora* Ehrenberg ex Kützing and treated as its subgenus. Both genera share many morphological features such as the shape of the valves and the characteristic amphoroidal shape of the entire cell, eccentric raphe systems, and areolae with external hymenes (Levkov 2009). The genus *Halamphora* was raised to the rank of an independent genus by Levkov (2009) and, currently this status is also justified by phylogenetic analyses based on molecular studies (Stepanek and Kociolek 2019; An et al. 2024; Yilmaz et al. 2024, 2025). The studies show that despite morphological similarity of the valves, *Amphora* and *Halamphora* are distantly related and do not represent the same family. The systematic position of *Halamphora* is not clear at this time. Although it is considered as a member of Amphipleuraceae (Kociolek et al. 2025; Guiry and Guiry 2026), phylogenetic studies show that *Halamphora* is a sister group to the raphid diatom clade that includes Rhopalodiales and Surirellales (Stepanek and Kociolek 2014).

Halamphora lukasiewiczii sp. nov. shows the greatest morphological similarity to the recently described *H. witkowskii*, which can be distinguished from *H. lukasiewiczii* sp. nov. mainly based on its lower stria density, both on the dorsal (20–22 in 10 µm vs. 24–30 in 10 µm) and ventral sides (ca. 32 versus ca. 55) (Yilmaz et al. 2025).

Great similarity also occurs between the newly described species and *Halamphora aponina*. Both species occur in brackish environments and share similar stria morphology (one to two rows of areolae on the dorsal side and a single row on the ventral side) and raphe ledge (almost linear and weakly expanded near the valve ends) (Levkov 2009). However, cells of *H. lukasiewiczii* sp. nov. are significantly shorter (8.0–22.5 µm vs. 23.0–40.0 µm length) and have higher stria density on the dorsal side (see Table 1) (Levkov 2009).

Despite its similar valve size to *H. kenderoviana*, *H. lukasiewiczii* sp. nov. can easily be distinguished based on the striae density on both valve sides. *Halamphora lukasiewiczii* sp. nov. has a higher striae density on both the dorsal and ventral sides (respectively: 24–30 in 10 µm and ca. 55 in 10 µm) compared to *H. kenderoviana* (respectively: 18–20 in 10 µm and 27–28 in 10 µm) (Zidarova et al. 2022). An additional difference is the structure of the striae on the ventral side, which consists of numerous areolae in *H. lukasiewiczii* sp. nov., while in *H. kenderoviana* the striae consist of only single elongated areolae (Zidarova et al. 2022).

Compared to the newly described species, *H. angustiformis* has lower striae density (20–22 in 10 µm on the dorsal side, 31–32 in 10 µm on the ventral side vs. 24–30 in 10 µm on the dorsal side, ca. 55 in 10 µm on the ventral side for *H. lukasiewiczii* sp. nov.). Furthermore, the striae on the dorsal side consist of only a single row of areolae in *H. angustiformis*, whereas *H. lukasiewiczii* sp. nov. has biseriate striae (Stepanek and Kociolek 2018). An additional difference is the structure of the striae on the ventral side of the frustule. *Halamphora angustiformis* has short striations composed of a single areola, while *H. lukasiewiczii* sp. nov. has striations composed of numerous small areolae (Stepanek and Kociolek 2018).

Another species that is similar in general appearance to *H. lukasiewiczii* sp. nov. is *H. borealis*. This species also presents similar ecological preferences

Table 1. Morphometric comparation of *Halamphora lukasiewiczii* sp. nov. with similar species.

	H. lukasiewiczii sp. nov.	*H. angustiformis*	*H. aponina*	*H. borealis*	*H. kenderoviana*	*H. witkowskii*	*H. margalefii* var. *lacustris*	
habitat	brackish to heavily salted with constant petroleum presence	freshwater	brackish/ marine	brackish	marine	brackish (soda lake)	freshwater	freshwater
valve length [µm]	8.0–22.5	15.0–33.0	23.0–40.0	19.0–40.0	13.5–20.5	14.0–31.0	18.0–27.0	15.0–18.0
valve width [µm]	3.0–5.0	3.0–4.0	3.0–4.5	3.0–4.0	3.5–4.5	4.0–6.0	3.1–4.9	2.5–3.0
striae on dorsal side [in 10 µm]	24–30	20–22	20–22	20–24	18–20	20–22	18–20	23–24
striae on ventral side [in 10 µm]	ca. 55	31–32	no data	no data	27–28	ca. 32*	no data	42–46
raphe ledge	well developed, almost linear and weakly expanded in the center and near the valve ends	well developed, almost linear and weakly expanded in the center and near the valve ends	narrow, almost linear and weakly expanded near valve ends	narrow, widened in the center and near valve ends	distinct, clearly widened in the valve middle, truncated and slightly expanded at the apices	well developed, almost linear*	well developed, almost linear*	very broad
central area	small, only on ventral side	only on ventral side	small fascia only on ventral side	wide fascia only on ventral side	on the dorsal side very small to usually absent, on the ventral side clearly enlarged	small, only on ventral side	only on ventral side	only on ventral side
references	this study	Stepanek and Kociolek 2018	Levkov 2009	Levkov 2009	Zidarova et al. 2022	Yilmaz et al. 2025	Sanchez-Castillo 1993	Stepanek and Kociolek 2018

* data obtained from photography in original description.

and of raphe ledge morphology to *H. lukasiewiczii* sp. nov., but it has longer valves (19.0–40.0 µm vs. 8.0–22.5 µm length) with lower stria density (20–24 in 10 µm vs. 24–30 in 10 µm) (Levkov 2009). Moreover, *H. borealis* has a larger central area and the striae are composed of a single row of areolae (Levkov 2009: plate 131, figs 1, 3, 4).

Despite similarities in valve morphology, the species mentioned above are nested inside separate phylogenetic clades, as previously defined by Stepanek and Kociolek (2019). Our results indicate that *H. lukasiewiczii* sp. nov. is positioned in Clade F with *H. bicapiatata*, *H. fontinalis*, *H. isumiensis*, *H. margalefii* var. *lacustris*, *H. nagumoi*, and *H. subturgida*. Based on previous studies, morphologically similar species *H. aponina*, *H. borealis*, and *H. witkowskii* are located in Clade D, Clade E, and Clade K, respectively (Stepanek and Kociolek 2019; Yilmaz et al. 2025). These clades, together with Clade F, include species common for both brackish and freshwater environments (Stepanek and Kociolek 2019).

Based on the phylogenetic analyses, *H. margalefii* var. *lacustris* is a sister taxon to *H. lukasiewiczii* sp. nov. Although the short branch lengths on every retrieved tree observed between *H. lukasiewiczii* sp. nov. and *H. margalefii* var. *lacustris* does not necessarily support species-level distinction, the two species are consistently retrieved separate and well supported (Fig. 2, Suppl. material 1: figs S1, S2). Additionally, both are readily distinguishable based on morphological features and their ecology. Compared to *H. lukasiewiczii* sp. nov., *H. margalefii* var. *lacustris* is a freshwater species (Sánchez-Castillo 1993;

Stepanek and Kociolek 2018). Based on data from the literature regarding both European (Spain) and North American populations of *H. margalefii* var. *lacustris*, this species is characterized by a lower stria density on their valves compared to *H. lukasiewiczii* sp. nov. (see Table 1). Moreover, *H. margalefii* var. *lacustris* has a wider raphe ledge in relation to the entire valve, which also has irregularly arranged pores on the dorsal side (Stepanek and Kociolek 2018: plate 53, figs 4, 5), while *H. lukasiewiczii* sp. nov. has no pores on the dorsal side of the raphe ledge.

Halamphora lukasiewiczii sp. nov. undoubtedly can be considered an extremophile species, as its natural habitat is water in natural oil seeps. Only one diatom species – *Navicula salinarum* – was found to co-occur with it. Observed valve morphology and dimensions were consistent with the literature data (Lange-Bertalot et al. 2017) as well as DNA sequences data, which place it together with another strain belonging to *N. salinarum* (99.82% similarity). This species occurs mainly off marine coasts, in intertidal zones of rivers, and is common in tidal marsh waters. In inland waters, it naturally inhabits salt-rich waters and moderately to strongly salinized freshwater habitats (Fukushima et al. 2013; Lange-Bertalot et al. 2017). Moreover, *Navicula salinarum* is found in inland brines with a high load of hydrocarbons of natural origin (Baker et al. 2022). The genus *Halamphora* is common in inland water bodies with high conductivity, often salt-rich and semiterrestrial habitats (Sala et al. 2007; Levkov 2009; You et al. 2015; Lange-Bertalot et al. 2017), but most frequently it occurs in marine and brackish habitats (Stepanek and Kociolek 2013, 2018). Similarly, high salinity conditions were documented at the sites studied, especially at site 2, where the conductivity from the salt (NaCl) content was extremely high (75400–81000 $\mu S \times cm^{-1}$). Observations in cultures also confirm that the species prefer to occur in highly conductivity (saline-rich) environments, and cells inoculated in freshwater medium did not show any signs of growth (authors' unpublished observation).

At first glance, natural oil seeps seem to be extreme environments that offer little to no chance for diatoms to survive because of the physicochemical conditions.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Mateusz Rybak, Łukasz Peszek and Ewa Górecka contributed equally to the work. Mateusz Rybak: Conceptualization, Data curation, Formal analysis, Funding acquisition Investigation, Methodology, Writing – original draft, Writing – review & editing. Łukasz Peszek: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. Ewa Górecka: Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing. Renata Zadrąg-Tęcza: Data curation, Writing – original draft. Zlatko Levkov: Writing – original draft , Writing – review & editing.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Phylogenetic trees

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Data type: docx

Explanation note: **fig. S1.** Maximum likelihood phylogenetic tree inferred from SSU rRNA gene sequences. Sequences belonging to *Halamphora lukasiewiczii* sp. nov. are indicated by box. ML bootstrap support (BP) below 50 are omitted. The scale bar represents 0.01 substitutions per site. Clades follow the classification proposed by Stepanek and Kociolek (2019). **fig. S2.** Maximum likelihood phylogenetic tree inferred from concatenated SSU rRNA and *rbc*L genes sequences. Sequences belonging to *Halamphora lukasiewiczii* sp. nov. are indicated by box. **fig. S3.** Maximum likelihood phylogenetic tree inferred from SSU rRNA gene sequences. Sequence belonging to *Navicula salinarum* is indicated by box. ML bootstrap support (BP) below 50 are omitted. The scale bar represents 0.01 substitutions per site.

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Supplementary material 2

List of sequences – supporting data

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Data type: zip

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